adding to the sample antibody molecules immunoreactive with a specific lipoprotein or apolipoprotein, wherein the antibody specifically binds to a stable, conformation independent epitope which is uninfluenced by the lipid content;

allowing the antibody molecules time to bind to the lipoprotein or apolipoprotein in the sample; and

determining the amount of lipoprotein, apolipoprotein, or lipid associated with a lipoprotein bound by the immobilized antibody molecules.

2. (Amended) The method of claim 1 wherein the antibody molecules are immunoreactive with a lipoprotein[s] selected from the group consisting of HDL, LDL, VLDL, and combinations thereof.

6. (Amended) The method of claim 1 wherein the antibodies are immobilized [into] onto a solid phase material, further comprising separating the solid phase material

13. (Amended) The method of claim 1 [for determining the concentration of an apolipoprotein in a biological sample] further comprising:

mixing an antibody immunoreactive with a specific apolipoprotein into the sample;

allowing the antibody to bind to the apolipoprotein in the sample,

[immersing into the mixture a second immobilized] adding to the mixture a second antibody immunoreactive with a second, distinct epitope of the apolipoprotein,

allowing the second immobilized antibody to bind to the apolipoprotein,

detecting the presence of the apolipoprotein bound by both antibodies, and

determining the amount of apolipoprotein bound by both antibodies.

15. (Amended) The method of claim 13 for determining the relative ratio of VLDL to HDL comprising

determining the amount of VLDL in a sample based on the amount of Apo C-III present in the VLDL in the sample by

providing Pan B [immobilized] antibody which is characterized by an equal binding and high affinity for all Apo B-containing lipoproteins in human plasma,

providing soluble antibody immunoreactive with Apo C-III having binding affinity and specificity similar to XbA_3,

mixing the soluble antibody reactive with Apo C-III with the biological sample to form complexes between the soluble antibody and the Apo C-III containing lipoprotein particles,

[immersing] adding the immobilized Pan B antibody to the biological sample, and

determining the amount of Apo C-III associated with Apo B, which is the amount of Apo C-III present in VLDL in the sample; and

determining the amount of HDL in a sample based on the amount of Apo C-III present in the HDL in the sample by

providing Apo A-I antibody immunoreactive specifically with Apo A-I having a binding affinity and specificity similar to $AIbD_5$ and $AIbE_2$,

providing soluble antibody immunoreactive with Apo C-III having binding affinity and specificity similar to XbA_3,

mixing the soluble antibody reactive with Apo C-III with the biological sample to form complexes between the soluble antibody and the Apo C-III containing lipoprotein particles,

immersing the immobilized anti-Apo A-I antibody into the biological sample, and

determining the amount of Apo C-III associated with Apo A-I, which is the amount of Apo C-III present in HDL in the sample.

16. (Amended) The method of claim 13 for determining the relative ratio of VLDL to HDL comprising

determining the amount of VLDL in a sample based on the amount of Apo E present in the VLDL in the sample by

providing [immobilized] Pan B antibody which is characterized by an equal binding and high affinity for all Apo B-containing lipoproteins in human plasma,

providing a mixture of soluble antibody immunoreactive with Apo E having binding affinity and specificity similar to EfB_1 which binds to Apo E associated predominantly with VLDL and soluble antibody immunoreactive with Apo E having

binding affinity and specificity similar to EfD_3 which binds to Apo E associated predominantly with HDL,

adding the mixture of soluble antibodies reactive with Apo E to the biological sample to form complexes between the soluble antibodies and Apo E containing particles,

immersing the immobilized Pan B antibody into the biological sample, and

determining the amount of Apo E associated with Apo B which is the Apo E present predominantly in VLDL in the sample; and

determining the amount of HDL in a sample based on the amount of Apo E present in the HDL in the sample by

providing [immobilized] Apo A-I antibody immunoreactive specifically with Apo A-I having a binding affinity and specificity similar to $AIbD_5$,

providing a mixture of soluble antibody immunoreactive with Apo E having binding affinity

and specificity similar to EfB_1, which binds to Apo E predominantly associated with VLDL, and soluble antibody immunoreactive with Apo E having binding affinity and specificity similar to EfD_3, which binds to Apo E predominantly associated with HDL,

adding the mixture of soluble antibodies reactive with Apo E to the biological sample to form complexes between the soluble antibodies and Apo E containing particles, and

determining the amount of Apo E associated with Apo A-I, which is the amount of Apo E present in HDL in the sample.

17. (Amended) The method of claim 13 for determining the relative ratio of LPA-I and LPA-II lipoprotein particles comprising

providing [immobilized] anti-Apo A-I antibody immunoreactive specifically with Apo A-I having a binding affinity and specificity similar to $AIbD_{5;}$,

providing [immobilized] anti-Apo A-II antibody immunoreactive specifically with Apo A-II having a binding affinity and specificity similar to CdB_5;

mixing the soluble anti-Apo A-I antibody having a binding affinity and specificity similar to $A1bE_2$ to form complexes with both LPA-I and LPA-I:AII;

immersing the [immobilized] anti-Apo A-I antibody into the biological sample and determining the quantity of Apo A-I associated with both LPA-I and LPA-II lipoprotein particles;

immersing the [immobilized] anti-Apo A-II antibody into the biological sample and determining the quantity of Apo A-I associated with the LPA-I:AII.

18. (Amended) A composition for determining the concentration of a lipoprotein, apolipoprotein, or lipid associated with a specific lipoprotein in a biological sample comprising:

a solid phase material having immobilized thereon antibody molecules specifically immunoreactive with a specific lipoprotein or apolipoprotein, wherein the antibody specifically binds to a stable, conformation independent epitope which is uninfluenced by the lipid content.

30. (Amended) The composition of claim 18 for determining the relative ratio of LPA-I and LPA-II lipoprotein particles comprising

immobilized Apo-A-I antibody which binds Apo A-I lipoproteins in human plasma having a binding affinity and specificity [similar to] with Apo $AIbD_5$; and

immobilized Apo A-II antibody immunoreactive specifically with Apo A-II having a binding affinity and specificity similar to CdB_5.

31. (Amended) A method for making a composition [for determining the concentration of a specific lipoprotein, an apolipoprotein, or lipid associated with a specific lipoprotein, in a biological sample] comprising

immobilizing on a solid phase material antibody molecules immunoreactive with a specific lipoprotein or apolipoprotein, wherein the antibody molecules are selected from the group consisting of monoclonal antibodies, recombinant antibodies, and fragments thereof, and wherein the antibody has a binding affinity of at least 10^9 for a stable, conformation independent epitope which is uninfluenced by the lipid content.

Please cancel claims 32-39 and replace with the following claims 32-44:

32. A method for making a composition for determining the concentration of a specific lipoprotein, an apolipoprotein, or lipid associated with a specific lipoprotein, in a biological sample comprising

immobilizing on a solid phase material antibody molecules immunoreactive with a specific lipoprotein or apolipoprotein, wherein the antibody molecules are selected from the group consisting of monoclonal antibodies, recombinant antibodies, and fragments thereof, and wherein the antibody specifically binds to a stable, conformationindependent epitope which is uninfluenced by the lipid content.

33. The method according to claim 32 wherein the antibody molecule is specifically immunoreactive with LDL.

34. The method of claim 32 wherein the apolipoprotein is selected from the group consisting of Apo A-I, Apo A-II, Apo B, Apo C-III, and Apo E.

35. An antibody molecule specifically immunoreactive with LDL that does not significantly cross-react with other lipoproteins in whole blood, blood plasma or blood serum, wherein the molecule is selected from the group consisting of monoclonal antibodies, recombinant antibodies, and fragments thereof and wherein the antibody specifically binds to a stable, conformation independent epitope which is uninfluenced by the lipid content.

36. The antibody molecule of claim 35 wherein the antibody is the anti-LDL monoclonal antibody produced by the hybridoma cell line HB_3cB_3 ATCC designation number HB 11612.

37. The antibody molecule of claim 35 wherein the antibody is a recombinant anti-LDL $RcB_3M_1D_4$ ATCC designation number 69602.

38. The antibody molecule of claim 35 immobilized to a solid support.

39. The antibody molecule of claim 38 wherein the support is a resin for purification of apolipoprotein, lipoprotein, or lipid associated therewith.

40. A method for purifying an apolipoprotein comprising

reacting a solution containing apolipoprotein with an immobilized antibody selected from the

group consisting of the anti-LDL monoclonal antibody produced by the hybridoma cell line HB_3cB_3
ATCC designation number HB 11612 and the anti-LDL $RcB_3M_1D_4$ recombinant antibody ATCC designation number 69602.

41. The method of claim 12 wherein binding of the second antibody forms a precipitate of the antigen and both bound antibodies which can be detected in solution.

42. The method of claim 1 for determining the relative ratio of LDL to HDL comprising

adding to the sample antibody molecules immunoreactive with low density lipoprotein and not cross-reactive with high density lipoprotein and determining the amount of low density lipoprotein;

adding to the sample antibody molecules immunoreactive with high density lipoprotein and not cross-reactive with low density lipoprotein and determining the amount of high density lipoprotein; and

determining the ratio of the amount of low density lipoprotein with the amount of high density lipoprotein.

43. The method of claim 41 wherein the anti-low density lipoprotein antibody is selected from the group consisting HB_3cB_3 and recombinant $RcB_3M_1D_4$ antibodies.

44. The method of claim 43 wherein the method comprises

(a) determining the amount of low density lipoprotein in a sample by

providing immobilized anti-LDL antibodies,

providing soluble labelled anti-ApoB antibodies,

mixing the soluble anti-ApoB antibody in the biological sample to form complexes between the soluble antibody and the ApoB containing lipoprotein particles,

determining the amount of ApoB captured by the immobilized antibody to calculate the amount of LDL,

(b) determining the amount of high density lipoprotein in a sample by

providing immobilized anti-ApoA-I antibody having a binding affinity and specificity similar to $AIbD_5$,

providing soluble labelled antibody having a binding affinity and specificity similar to $AIbE_2$,

mixing the soluble antibody reactive with ApoA-I in the biological sample to form complexes between the soluble antibody and the ApoA-I in lipoprotein particles,

determining the amount of ApoA-I captured by the immobilized antibody and calculating the amount of HDL from the amount of ApoA-I, and

(c) calculating the ratio of LDL to HDL.

Respectfully submitted,

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Date: 24 December 1996

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CERTIFICATE OF MAILING UNDER 37 CFR § 1.10

I hereby certify that this Preliminary Amendment and any documents referred to as attached therein are being deposited with the United States Postal Service on this date, 24 December 1996, in an envelope as "Express Mail Post Office to Addressee" service under 37 CFR § 1.10, Mailing Label Number EM470184783US addressed to Box PCT, Assistant Commissioner for Patents, Washington, D.C. 20231.

Date: 24 December 1996

Robert J. Copeland

OMRF143 CIP(2)
Pending Claims

1. A method for determining the concentration of a specific lipoprotein, an apolipoprotein, or lipid associated with a specific lipoprotein, in a biological sample comprising:

adding to the sample antibody molecules immunoreactive with a specific lipoprotein or apolipoprotein, wherein the antibody specifically binds to a stable, conformation independent epitope which is uninfluenced by the lipid content;

allowing the antibody molecules time to bind to the lipoprotein or apolipoprotein in the sample; and

determining the amount of lipoprotein, apolipoprotein, or lipid associated with a lipoprotein bound by the immobilized antibody molecules.

2. The method of claim 1 wherein the antibody molecules are immunoreactive with a lipoprotein selected from the group consisting of HDL, LDL, VLDL, and combinations thereof.

3. The method of claim 2 wherein the antibody is selected from the group consisting of monoclonal antibodies, recombinant antibodies, and antibody fragments.

4. The method of claim 3 wherein the antibody is the anti-LDL monoclonal antibody produced by the hybridoma cell line HB_3cB_3 ATCC designation number HB 11612.

5. The method of claim 3 wherein the antibody is a recombinant anti-LDL $RcB_3M_1D_4$ ATCC designation number 69602.

6. The method of claim 1 wherein the antibodies are immobilized onto a solid phase material, further comprising separating the solid phase material containing the immobilized antibody molecules from the biological sample.

7. The method of claim 1, wherein the amount of lipoprotein, apolipoprotein lipid is determined by staining of the material bound to the immobilized antibody using a lipid stain.

8. The method of claim 7 wherein the immobilized antibodies are immersed into the biological sample and the lipoprotein lipid is stained prior to immersing the immobilized antibodies.

9. The method of claim 8 further comprising antibody immunoreactive with apolipoprotein which is coupled to a protein stain and used to stain lipoprotein in the sample, prior to immersing into the sample the immobilized antibodies which then bind to the stained antibody-bound apolipoprotein.

10. The method of claim 1 wherein the apolipoprotein is selected from the group consisting of Apo A-I, Apo A-II, Apo B, Apo C-III, and Apo E.

11. The method of claim 1 wherein the biological sample is selected from the group consisting of blood, plasma, and serum.

12. The method of claim 1 wherein the assay is a turbidimetric assay.

13. The method of claim 1 further comprising:
mixing an antibody immunoreactive with a specific apolipoprotein into the sample;
allowing the antibody to bind to the apolipoprotein in the sample,
adding to the mixture a second antibody immunoreactive with a second, distinct epitope of the apolipoprotein,
allowing the second immobilized antibody to bind to the apolipoprotein,
detecting the presence of the apolipoprotein bound by both antibodies, and
determining the amount of apolipoprotein bound by both antibodies.

14. The method of claim 13 wherein the apolipoprotein is apolipoprotein Apo B-100.

15. The method of claim 13 for determining the relative ratio of VLDL to HDL comprising

determining the amount of VLDL in a sample based on the amount of Apo C-III present in the VLDL in the sample by

providing Pan B antibody which is characterized by an equal binding and high affinity for all Apo B-containing lipoproteins in human plasma,

providing soluble antibody immunoreactive with Apo C-III having binding affinity and specificity similar to XbA_3,

mixing the soluble antibody reactive with Apo C-III with the biological sample to form complexes between the soluble antibody and the Apo C-III containing lipoprotein particles,

adding the immobilized Pan B antibody to the biological sample, and

determining the amount of Apo C-III associated with Apo B, which is the amount of Apo C-III present in VLDL in the sample; and

determining the amount of HDL in a sample based on the amount of Apo C-III present in the HDL in the sample by

providing Apo A-I antibody immunoreactive specifically with Apo A-I having a binding affinity and sp
ecificity similar to $AIbD_5$ and $AIbE_2$,

providing soluble antibody immunoreactive with Apo C-III having binding affinity and specificity similar to XbA_3,

mixing the soluble antibody reactive with Apo C-III with the biological sample to form complexes between the soluble antibody and the Apo C-III containing lipoprotein particles,

immersing the immobilized anti-Apo A-I antibody into the biological sample, and

determining the amount of Apo C-III associated with Apo A-I, which is the amount of Apo C-III present in HDL in the sample.

16. The method of claim 13 for determining the relative ratio of VLDL to HDL comprising

determining the amount of VLDL in a sample based on the amount of Apo E present in the VLDL in the sample by

providing Pan B antibody which is characterized by an equal binding and high affinity for all Apo B-containing lipoproteins in human plasma,

providing a mixture of soluble antibody immunoreactive with Apo E having binding affinity and specificity similar to EfB_1 which binds to Apo E associated predominantly with VLDL and soluble antibody immunoreactive with Apo E having binding affinity and specificity similar to EfD_3 which binds to Apo E associated predominantly with HDL,

adding the mixture of soluble antibodies reactive with Apo E to the biological sample to form complexes between the soluble antibodies and Apo E containing particles,

immersing the immobilized Pan B antibody into the biological sample, and

determining the amount of Apo E associated with Apo B which is the Apo E present predominantly in VLDL in the sample; and

determining the amount of HDL in a sample based on the amount of Apo E present in the HDL in the sample by

providing Apo A-I antibody immunoreactive specifically with Apo A-I having a binding affinity and specificity similar to $AIbD_5$,

providing a mixture of soluble antibody immunoreactive with Apo E having binding affinity
and specificity similar to EfB_1, which binds to Apo E predominantly associated with VLDL, and soluble antibody immunoreactive with Apo E having binding affinity and specificity similar to EfD_3, which binds to Apo E predominantly associated with HDL,

adding the mixture of soluble antibodies reactive with Apo E to the biological sample to form complexes between the soluble antibodies and Apo E containing particles, and

determining the amount of Apo E associated with Apo A-I, which is the amount of Apo E present in HDL in the sample.

17. The method of claim 13 for determining the relative ratio of LPA-I and LPA-II lipoprotein particles comprising

providing anti-Apo A-I antibody immunoreactive specifically with Apo A-I having a binding affinity and specificity similar to $AIbD_{5;}$,

providing anti-Apo A-II antibody immunoreactive specifically with Apo A-II having a binding affinity and specificity similar to CdB_5;

mixing the soluble anti-Apo A-I antibody having a binding affinity and specificity similar to $A1bE_2$ to form complexes with both LPA-I and LPA-I:AII;

immersing the anti-Apo A-I antibody into the biological sample and determining the quantity of Apo A-I associated with both LPA-I and LPA-II lipoprotein particles;

immersing the anti-Apo A-II antibody into the biological sample and determining the quantity of Apo A-I associated with the LPA-I:AII.

18. A composition for determining the concentration of a lipoprotein, apolipoprotein, or lipid associated with a specific lipoprotein in a biological sample comprising:

a solid phase material having immobilized thereon antibody molecules specifically immunoreactive with a specific lipoprotein or apolipoprotein, wherein the antibody specifically binds to a stable, conformation independent epitope which is uninfluenced by the lipid content.

19. The composition of claim 18 further comprising a solid support to which the solid phase material is attached to form a dipstick.

20. The composition of claim 18 wherein the antibody is selected from the group consisting of monoclonal antibodies, recombinant antibodies, and antibody fragments.

21. The composition of claim 18 wherein the antibody is the anti-LDL monoclonal antibody produced by the hybridoma cell line HB_3cB_3 ATCC designation number HB 11612.

22. The composition of claim 18 wherein the antibody is a recombinant anti-LDL $RcB_3M_1D_4$ ATCC designation number 69602.

23. The composition of claim 18 further comprising a solution containing molecules of a second soluble antibody immunoreactive with a second distinct epitope of the lipoprotein or apolipoprotein which is immunoreactive with the antibody molecules immobilized on the solid phase material.

24. The composition of claim 18 wherein the antibody molecules are immobilized to the solid phase material using avidin-biotin complexes.

25. The composition of claim 19 further comprising at least one internal standard comprising a known amount of a particular lipoprotein, lipoprotein lipid, or apolipoprotein immobilized on the solid phase material.

26. The composition of claim 18 wherein the solid phase material is selected from the group consisting of nitrocellulose, polyvinylidene difluoride, partially acid-hydrolyzed nylon, polystyrene, polypropylene, and paper.

27. The composition of claim 18 wherein the apolipoprotein is selected from the group consisting of Apo A-I, Apo A-II, Apo B, Apo C-III, and Apo E.

28. The composition of claim 18 for determining the relative ratio of VLDL to HDL comprising

immobilized Pan B antibody which is characterized by an equal binding and high affinity for all Apo B-containing lipoproteins in human plasma,

soluble antibody immunoreactive with Apo C-III having binding affinity and specificity similar to XbA_3,

immobilized Apo A-I antibody immunoreactive specifically with Apo A-I having a binding affinity and specificity similar to $AIbD_5$ and $AIbE_2$, and

soluble antibody immunoreactive with Apo C-III having binding affinity and specificity similar to XbA_3.

29. The composition of claim 18 for determining the relative ratio of VLDL to HDL comprising

immobilized Pan B antibody which is characterized by an equal binding and high affinity for all Apo B-containing lipoproteins in human plasma,

a mixture of soluble antibody immunoreactive with Apo E having binding affinity and specificity similar to EfB_1 which predominantly binds to Apo E associated with VLDL and soluble antibody immunoreactive with Apo E having binding affinity and specificity similar to EfD_3 which predominantly binds to Apo E in HDL,

immobilized Apo A-I antibody immunoreactive specifically with Apo A-I having a binding affinity and specificity similar to $AIbD_5$, and

a mixture of soluble antibody immunoreactive with Apo E having binding affinity and specificity
similar to EfB_1 which binds to Apo E predominantly associated with VLDL and soluble antibody immunoreactive with Apo E having binding affinity and specificity similar to EfD_3 which predominantly binds to Apo E in HDL.

30. The composition of claim 18 for determining the relative ratio of LPA-I and LPA-II lipoprotein particles comprising

immobilized Apo-A-I antibody which binds Apo A-I lipoproteins in human plasma having a binding affinity and specificity with Apo $AIbD_5$; and

immobilized Apo A-II antibody immunoreactive specifically with Apo A-II having a binding affinity and specificity similar to CdB_5.

31. A method for making a composition comprising

immobilizing on a solid phase material antibody molecules immunoreactive with a specific lipoprotein or apolipoprotein, wherein the antibody molecules are selected from the group consisting of monoclonal antibodies, recombinant antibodies, and fragments thereof, and wherein the antibody has a binding affinity of at least 10^9 for a stable, conformation independent epitope which is uninfluenced by the lipid content.

32. A method for making a composition for determining the concentration of a specific lipoprotein, an apolipoprotein, or lipid associated with a specific lipoprotein, in a biological sample comprising

immobilizing on a solid phase material antibody molecules immunoreactive with a specific lipoprotein or apolipoprotein, wherein the antibody molecules are selected from the group consisting of monoclonal antibodies, recombinant antibodies, and fragments thereof, and wherein the antibody specifically binds to a stable, conformationindependent epitope which is uninfluenced by the lipid content.

33. The method according to claim 32 wherein the antibody molecule is specifically immunoreactive with LDL.

34. The method of claim 32 wherein the apolipoprotein is selected from the group consisting of Apo A-I, Apo A-II, Apo B, Apo C-III, and Apo E.

35. An antibody molecule specifically immunoreactive with LDL that does not significantly cross-react with other lipoproteins in whole blood, blood plasma or blood serum, wherein the molecule is selected from the group consisting of monoclonal antibodies, recombinant antibodies, and fragments thereof and wherein the antibody specifically binds to a stable, conformation independent epitope which is uninfluenced by the lipid content.

36. The antibody molecule of claim 35 wherein the antibody is the anti-LDL monoclonal antibody produced by the hybridoma cell line HB_3cB_3 ATCC designation number HB 11612.

37. The antibody molecule of claim 35 wherein the antibody is a recombinant anti-LDL $RcB_3M_1D_4$ ATCC designation number 69602.

38. The antibody molecule of claim 35 immobilized to a solid support.

39. The antibody molecule of claim 38 wherein the support is a resin for purification of apolipoprotein, lipoprotein, or lipid associated therewith.

40. A method for purifying an apolipoprotein comprising

reacting a solution containing apolipoprotein with an immobilized antibody selected from the

group consisting of the anti-LDL monoclonal antibody produced by the hybridoma cell line HB_3cB_3
ATCC designation number HB 11612 and the anti-LDL $RcB_3M_1D_4$ recombinant antibody ATCC designation number 69602.

41. The method of claim 12 wherein binding of the second antibody forms a precipitate of the antigen and both bound antibodies which can be detected in solution.

42. The method of claim 1 for determining the relative ratio of LDL to HDL comprising

adding to the sample antibody molecules immunoreactive with low density lipoprotein and not cross-reactive with high density lipoprotein and determining the amount of low density lipoprotein;

adding to the sample antibody molecules immunoreactive with high density lipoprotein and not cross-reactive with low density lipoprotein and determining the amount of high density lipoprotein; and

determining the ratio of the amount of low density lipoprotein with the amount of high density lipoprotein.

43. The method of claim 41 wherein the anti-low density lipoprotein antibody is selected from the group consisting HB_3cB_3 and recombinant $RcB_3M_1D_4$ antibodies.

44. The method of claim 43 wherein the method comprises

(a) determining the amount of low density lipoprotein in a sample by

providing immobilized anti-LDL antibodies,

providing soluble labelled anti-ApoB antibodies,

mixing the soluble anti-ApoB antibody in the biological sample to form complexes between the soluble antibody and the ApoB containing lipoprotein particles,

determining the amount of ApoB captured by the immobilized antibody to calculate the amount of LDL,

(b) determining the amount of high density lipoprotein in a sample by

providing immobilized anti-ApoA-I antibody having a binding affinity and specificity similar to $AIbD_5$,

providing soluble labelled antibody having a binding affinity and specificity similar to $AIbE_2$,

mixing the soluble antibody reactive with ApoA-I in the biological sample to form complexes between the soluble antibody and the ApoA-I in lipoprotein particles,

determining the amount of ApoA-I captured by the immobilized antibody and calculating the amount of HDL from the amount of ApoA-I, and

(c) calculating the ratio of LDL to HDL.